Yeast Cell Lysis

Introduction

Preparation of cell extracts. Keep samples on ice at all times to avoid degradation

Materials

- Glass beads
- Sample buffer
- Protease inhibitor
- PMSF

Procedure

Lysis

1. Transfer culture to falcon tubes, put on ice
2. Spin down cells, 5-40 min, 3124 g, + 4 C

   3900 rpm in our centrifuge
3. Collect supernatant, resuspend pellet in H2O (0.05 OD/uL)

   H2O to wash the cells
4. Transfer suspension to an eppendorf tube, spin down for 5 minutes, 5000 g, +4 C
5. Resuspend pellet in sample buffer (w/o DTT) containing protease inhibitor and 1 mM PMSF

   PMSF breaks down quickly in RT or in water!
   Buffer can be be SDS-PAGE sample buffer or any other buffer
6. Add half the volume of glass beads
7. Vortex for 10 minutes at full speed in the cold room
8. Spin down samples at full speed for 5 minutes at +4 C to remove foam
9. Take out supernatant (around 100 uL) to new eppendorf

   (To do also a reduced sample for SDS-PAGE, take 47.5 uL supernatant and mix it with 2.5 uL 1 M DTT)
10. (To proceed with SDS-PAGE, boil samples for 5 minutes at 65 C)